SPINNERET SILK SPIGOT MORPHOLOGY:
EVIDENCE FOR THE MONOPHYLY OF ORBWEAVING SPIDERS, CYRTOPHORINAE (ARANEIDAE), AND THE GROUP THERIDIIDAE PLUS NESTICIDAE

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ABSTRACT

Labelled scanning electron micrographs comprehensively illustrating the spinning fields of *Deinopis* (Deinopidae), *Octonoba* (Uloboridae), *Araneus* (Araneidae), *Leucauge* (Tetragnathidae), *Latrodectus* and *Theridula* (Theridiidae), *Gaucelmus* (Nesticidae), and *Frontinella* (Linyphiidae) are presented for the first time. Evidence from scanning electron micrographs supports the monophyly of orbweavers (Orbiculariae = Uloboridae, Deinopidae, Araneoidea), the araneid subfamily Cyrtophorinae and the close relationship between Nesticidae and Theridiidae. This evidence is presented in the context of guidelines for the logical taxonomic interpretation of spider spigot morphology. This morphological system, including gross and detailed morphology, location and number of spigots, and serial homology relationships, may be one of the most complex, yet under-utilized, taxonomic character systems in spiders.

INTRODUCTION

Spinnerets both define and epitomize spiders. The history of studies on spider silk glands has been reviewed by Kovoor (1977b). Despite her elegant studies (e.g., Kovoor 1972, 1977a, 1977c, 1988; Kovoor and Lopez 1982; Kovoor and Peters 1988), filled with interesting facts about peculiar spinneret morphologies, systematists rarely report on this diverse character system in their phylogenetic studies. Several explanations for this neglect suggest themselves, such as belief that histological evidence on glands is essential for interpretation of the morphology, or that one must fix living material to obtain satisfactory results. Perhaps the chief difficulty is that a detailed histological study is so time consuming that a large diversity of species within a taxon cannot easily be surveyed. Variation thus remains undocumented, and taxonomists remain uncertain about the constancy of a feature, and the pattern of its distribution. However, on close inspection, none of these objections are insuperable. This paper outlines how spigot morphology can be studied using ordinary museum material. It presents some of the more interesting results so far, and summarizes a conceptual framework for the interpretation of the data that, I hope, will encourage the use of spigot morphology in spider systematics.

In this study I present preliminary results from a larger study on silk spigot diversity in spiders in general. The taxa included herein have been chosen to illustrate how silk spigot characters may bear on particular systematic problems, and to illustrate the diversity of silk spigot morphology. The specific systematic problems discussed are the monophyly of orbweavers, the monophyly of Cyrtophorinae (*Cyrtophora* and *Mecynogea*, at least), and the monophyly of a group including Theridiidae and Nesticidae. Although the evidence in all three cases seems positive, one must be cautious until further taxa are surveyed.

I have previously reviewed the evidence for the monophyly of Araneoidea and Deinopoidea (=Uloboridae and Deinopidae; Coddington 1986a, b). The evidence formerly believed to support the polyphyly of orbweavers was largely due to a confusion between symplesiomorphy and synapomorphy—the misinterpretation of primitive features that "defined" the symplesiomorphic group "Cribellatae." When the features that once defined the Cribellatae are recognized as primitive, there are no credible synapomorphies that place Deinopoidea with the rest of the cribellates, rather than with the other orbweavers (Araneoidea). On the contrary, most of the evidence implicates them as the sister taxon of the ecribellate Araneoidea. However, contrasting points of view have subsequently been expressed by Eberhard (1987), Shear (1986), Kovoor and Peters (1988) and Tyshchenko (1986).

The monophyly of the Cyrtophorinae, on the other hand, although never explicitly justified, has rarely been doubted (Levi 1980, 1983; Levi and Coddington 1983). This paper offers evidence independent of genitalic morphology and web architecture to confirm cyrtophorine monophyly.

The composition of the Theridiidae has frequently been questioned, as well as its relationship to Nesticidae. For example, Lehtinen and Saaristo (1980) placed the latter two families in different superfamilies, mainly because of genitalic differences. In particular they suggested that the fourth tarsal comb of serrated bristles common to both families was "purely adaptive." Palp and epigynal morphology among theridiids and nesticids obviously is diverse, but in any case offers no evidence to ally either group more closely with other araneoid taxa than with each other. However, the fourth tarsal comb is part of a behavior-morphology complex that enables nesticids and theridiids to subdue their prey with viscid sticky silk (Whitehouse 1987). This attack behavior is unique among spiders and stands as a strong synapomorphy of theridiids and nesticids (Coddington 1986a). This paper offers additional morphological evidence concordant with the fourth tarsal comb and the attack behavior itself. It is therefore additional evidence in favor of the monophyly of Nesticidae and Theridiidae.

This study also outlines the analytical methods one can use to deduce homologies among spigots, and thereby to use the data in phylogenetic analysis. Basically, Remane's criteria of homology (position, special similarity, ontogeny) seem entirely adequate, and thus the comparative study of silk spigots can proceed to some extent independently of other lines of evidence, such as histology or histochemistry. This is not to say that the spigot evidence is superior to histological evidence, but only that the morphology and exact location of araneomorph spigots seems complex enough and consistent enough to support generalizations. Histology is not *required* in order to infer spigot homologies. Spigot morphology may even help to decide questions of homology when the

histological evidence is equivocal. Indeed, when patterns in histological and histochemical data (e.g., Kovoor 1987) are compared with well-corroborated groups in spider phylogeny, many details of histochemical reactions, gland ultrastructure, and gland cell type are apparently homoplasious (see Discussion).

MATERIALS AND METHODS

Spigot morphology is extremely difficult to see with light microscopy. If one must use the light microscope, the best results are obtained with epi-illumination, but even then distinctive details are easy to miss (e.g., Mikulska 1966, 1967; Wasowska 1966, 1970). No doubt that helps to explain the neglect of spigot morphology as a character system in the past. However, the increasing availability of scanning electron microscopes (SEM) puts study of spigot morphologies well within the grasp of most spider taxonomists.

The best preparations have the spinnerets widely spread, are clean, and can be scanned from all angles. Several workers with whom I have spoken have had difficulties in getting good results, usually because the spinnerets are contracted upon themselves so that the distal articles cannot be seen, or else the spigots are covered with debris. In this study, these obstacles were overcome with good success by the following techniques.

Selection of material.—Obviously fresh material is best, although I have successfully prepared 50-100 year old specimens. If live material is available, kill the animals by direct, sudden immersion in boiling water or fixative. Under these conditions, the spinnerets are widely spread. In the case of ordinary museum material, the only real requirements are an intact set of spinnerets and a flexible abdomen. The latter is important in case the spinneret tips need to be spread. However, even hardened material can be used if one digests the spinneret group in a trypsin solution prior to mounting. All the material here is from the USNM collection, and voucher specimens are deposited there. Except in one case, only adult females were used.

Forceps squeeze.—As long as the abdomen of the specimen is still flexible, the spinnerets can be spread with forceps. This technique has been modified from one originally suggested by Dr. J. Kovoor. One must use cross-action forceps or otherwise be able to lock them in a closed position. One can use a rubber band around the forceps blades, or a wire collet that can slide forward to lock the tips. By experimenting with various angles, one can usually squeeze the abdomen immediately above (dorsal or anterior to) the spinnerets so that all six spinnerets spread widely. At this point, lock the forceps shut, sever the spinnerets from the abdomen with a razor, and run the spinnerets through an alcohol series up to anhydrous ethanol while they are still grasped by the forceps. Dehydration stiffens the spinnerets so that one can remove the forceps and the spinnerets will stay in a spread position. At this point, they are ready for cleaning.

If the forceps technique does not separate the spinnerets, sever the spinneret group and digest it completely in trypsin solution. This removes all muscle tissue and leaves only the cuticle of the spinneret group, so that it will be flexible enough to spread the spinnerets during the mounting process.

Ultrasonic cleaning.—Immersing the spinnerets (still gripped in the forceps) in an ultrasonic cleaner for 1-10 minutes will remove debris. If the specimen is

extremely fragile (e.g., Ochyroceratidae, Nesticidae, Symphytognathidae), decrease the time. Clean spigots are distinctly visible at 100X. Special care must be taken to clean trypsin-digested spinnerets. Brief soaking in 10% KOH to remove enzymatic protein is often helpful.

Stub mounting.—The cleaned, spread spinneret group is now ready to be mounted. One must use a stub so that the spinneret group can be rotated into nearly any angle for viewing. Standard, disc-shaped SEM stubs do not work well because the stub edge obscures the view or ruins the background contrast. I use 1/8 in. diameter steel rivets. The hemispherical head permits an unobstructed view of the spinnerets from any angle, and one can tip the stub to a 90° position so that the SEM background will be completely black. Spinnerets prepared by the forceps technique can be mounted with the usual adhesives, such as silver paint.

Trypsin-digested spinnerets that require spreading should be mounted with a stickier adhesive, such as the gum from double stick tape. In this case, the cured surface of the rivet anchors the outer edge of the cuticle, and by careful additional pushing and denting of the cuticle surface, one can spread the spinneret tips at this point.

RESULTS

Figure 1 diagrams the typical distribution of spigots in an araneoid spider. Figures 2-41 illustrate the diversity of spigots in *Deinopis* (Deinopidae), *Octonoba* (Uloboridae), *Araneus* (Araneidae), *Leucauge* (Tetragnathidae), *Cyrtophora* and *Mecynogea* (Araneidae: Cyrtophorinae), *Latrodectus* and *Theridula* (Theridiidae), *Gaucelmus* (Nesticidae), and *Frontinella* (Linyphiidae). Spigots in all figures are labelled in accordance with Table 1. Each plate of figures is laid out the same way. The upper left micrograph shows the left three spinnerets. Anterior is always at the top. The upper right micrograph shows the left ALS (anterior lateral spinneret) tip; the lower left micrograph the left PMS (posterior median spinneret) tip; and the lower right micrograph the left PLS (posterior lateral spinneret) tip. In the three close-up micrographs anterior is usually at the top or left, but if not, the orientation can be figured out by referring to the upper left micrograph. Abbreviations of spinneret and spigot terminology are given in the legend to Figs. 2-5. Spigots in Figs. 2-9 are labelled with arrows for precision, thereafter the labels are adjacent to the spigots.

Deinopoidea.—The cribellate *Deinopis* and *Octonoba* illustrate the orb-weaver ground plan. The piriform spinning field is more or less uniformly distributed across the ALS tip (Figs. 3, 7; PI). The major ampullate spigot(s) is (are) on the mesal margin of the ALS (Figs. 3, 7; MAP). Like the Araneoidea, adult female *Octonoba* have one functional major ampullate spigot, bordered posteriorly by a vestigial nubbin (also diagrammed in Fig. 1), presumably the remainder of the second MAP present in juveniles. *Deinopis* have multiple MAPs, a fairly rare feature among araneomorph spiders. The cuticle sculpturing is grooved and fluted (see also Kovoor and Peters 1988), apparently a synapomorphy for Neocribellatae.

The posterior median spinneret of *Octonoba* shows four classes of spigots. There are three cylindrical spigots and one posterior minor ampullate spigot (Fig.
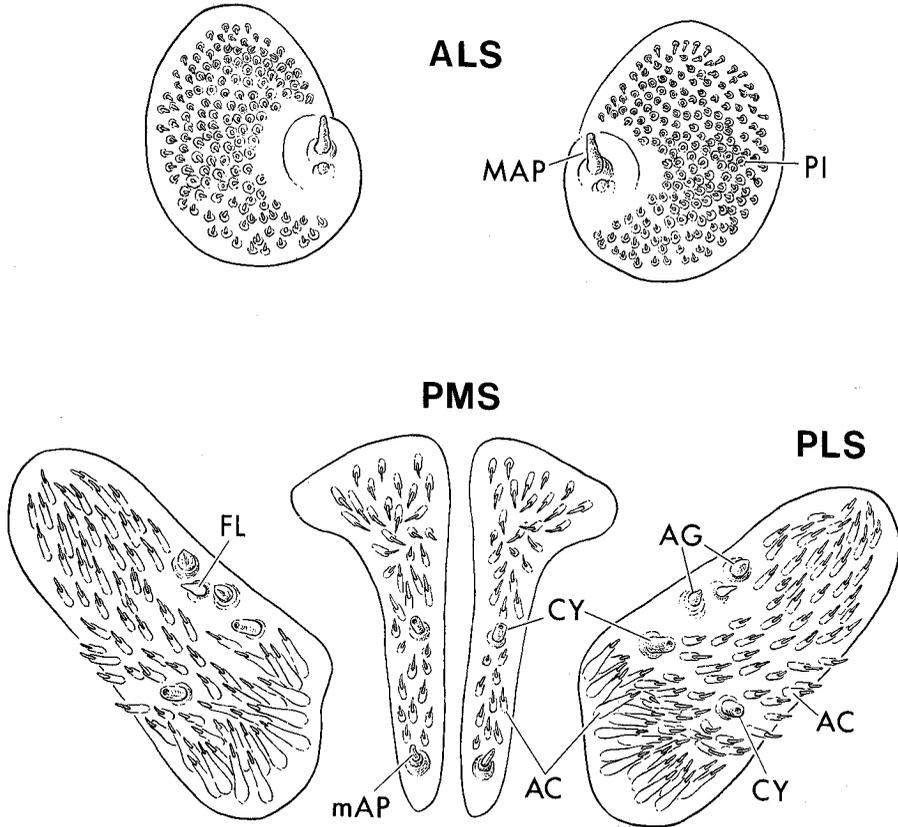
Figure 1.—Generalized araneoid spinneret and spigot location.

8; CY, mAP). Around and between them is a group of many (>30) small aciniform spigots (Fig. 8; AC), which extend forward on to the anterior wall of the PMS spinneret. Finally, there is an anterior brush of many elongate, annulated, paracribellar spigots (Fig. 8; PA). The condition in *Deinopis* is similar, although they seem to have many more cylindrical gland spigots than uloborids (Fig. 4; CY).

The posterior lateral spinneret is also complex. On the mesal basal margin in *Octonoba* are six cylindrical spigots (Fig. 9; CY). On the anterolateral margin is the pseudoflagelliform spigot (Fig. 9; PF). Distributed across the face of the PLS are a second group of aciniform spigots (Fig. 9; AC). Figure 5 shows a *Deinopis* immature female—thus she lacks all cylindrical spigots, and has only the pseudoflagelliform and aciniform spigots (Fig. 5; PF, AC,). Adult female *Deinopis*, like other deinopoids, have multiple, basal, cylindrical gland spigots on the PLS.

Araneoidea.—The spinning fields of *Araneus* illustrate the rather conservative and consistent araneoid ground plan. The cuticle sculpturing is lenticular or squamate, rather than fluted or grooved. That feature, of course, is found in other spider taxa than Araneoidea. As in Deinopoidea, the piriform spinning field is uniform across the ALS tip (e.g., Figs. 11, 23, 27, 31, 35, 39; PI). This condition has been distinctively modified in *Mecynogea* and *Cyrtophora*, however, whose piriform field has been restricted posteriorly to a ribbon (Figs.

Table 1.—Orbweaver silk spigots categorized by number (singular or multiple), the glands they presumably serve, and position in the spinneret field.

Spigot type	Position/Description
SINGULAR	
1. Major ampullate	A single spigot on the mesal border of the ALS. Present in most if not all araneomorphs.
2. Minor ampullate	A single spigot on the posterior margin of the PMS. Present in most if not all araneomorphs.
3. Cylindrical	In Araneoidea, 3 spigots: 2 on basal margin of PLS tip and 1 on anterior margin of PMS. Number varies in other groups, but apparently always on PMS and PLS.
4. Flagelliform	In Araneoidea, a unique spigot between the aggregate spigots of the PLS.
5. Pseudoflagelliform	One spigot on the antero-lateral margin of the PLS in some cribellate spiders; homologue of the flagelliform in araneoids?
6. Aggregate	In Araneoidea, two similar spigots near the flagelliform spigot.
MULTIPLE	
7. Piriform	A group of small apiculate spigots on the ALS. Present in most if not all araneomorphs; morphology but not position variable.
8. Aciniform	The most numerous gland type; small spigots present in multiples on PMS and PLS. Present in most if not all araneomorphs; morphology variable.
9. Paracribellar	A group of long, thin, often annulated spigots on the anterior PMS margin in some cribellate spiders.

15, 19; Pl). The major ampullate spigot with its vestigial partner is on the mesal margin of the ALS in all the araneoids (Figs. 11, 15, 19, 23, etc.; MAP).

The posterior median spinneret shows three classes of spigots: the single anterior cylindrical spigot, the single posterior minor ampullate spigot, and the surrounding and/or intervening group of many small aciniform spigots (Figs. 12, 16, 20, 24, 28, 32, 36, 40; CY, mAP, AC, respectively). In *Araneus* (Fig. 12), *Mecynogea* (Fig. 16), and *Cyrtophora* (Fig. 20), as in cribellate orbweavers, the aciniform spigot field extends forward on the anterior wall of the spinneret. The restriction of the PMS aciniform field to the spinneret tip, or at least its absence from the anterior face in *Leucauge* (Fig. 24), and other derived araneoids such as theridiids (Figs. 28, 32), nesticids (Fig. 36), and linyphiids (Fig. 40), is a derived condition. *Leucauge, Latrodectus,* and *Gaucelmus* have just three aciniform spigots, *Theridula* and *Frontinella* (as well as other linyphiids) have only two.

In Araneoidea, the posterior lateral spinneret is the most complex. On its mesal basal margin are two cylindrical spigots (e.g., Figs. 13, 25; CY), although their position sometimes shifts (e.g., *Frontinella,* Fig. 41). On the anterolateral margin is the flagelliform, and the two aggregate glue gland spigots (e.g., Figs. 13, 25, 41; FL, AG). Distributed across the PLS tip is a second group of aciniform spigots (e.g., Figs. 17, 21; AC). The PLS flagelliform-aggregate complex in *Cyrtophora* is entirely absent, whereas it is merely reduced in *Mecynogea* (compare Figs. 17, 21 with "normal" spigots in *Araneus,* Fig. 13, or *Frontinella,* Fig. 41).

The theridiid and nesticid complements are also modified, but in a different way. On the theridiid PLS, the two aggregate glands are relatively enormous, the ectal larger than the mesal (e.g., *Latrodectus, Theridula* Figs. 29, 33; AG). The same situation occurs in nesticids, although the difference between the two
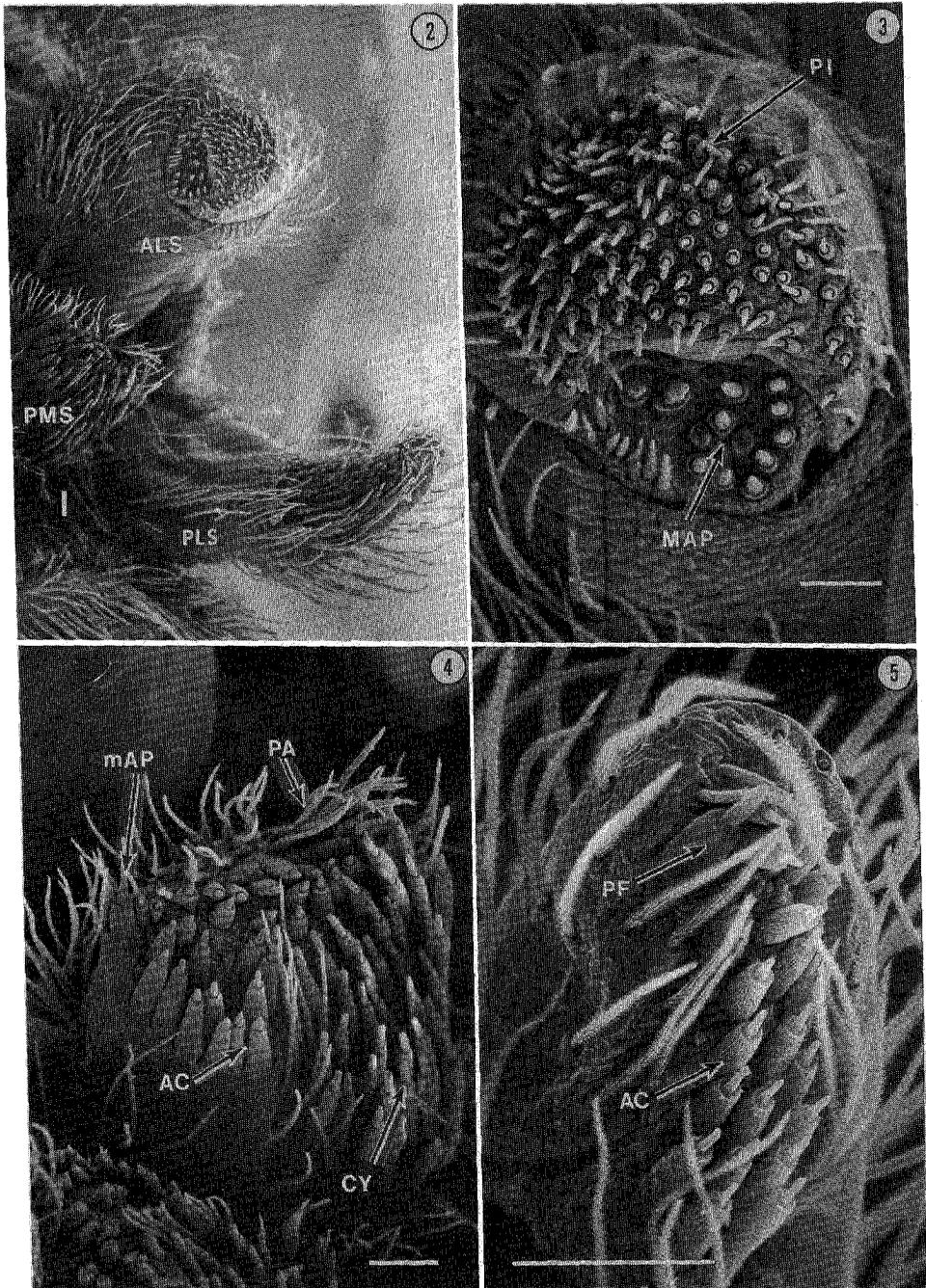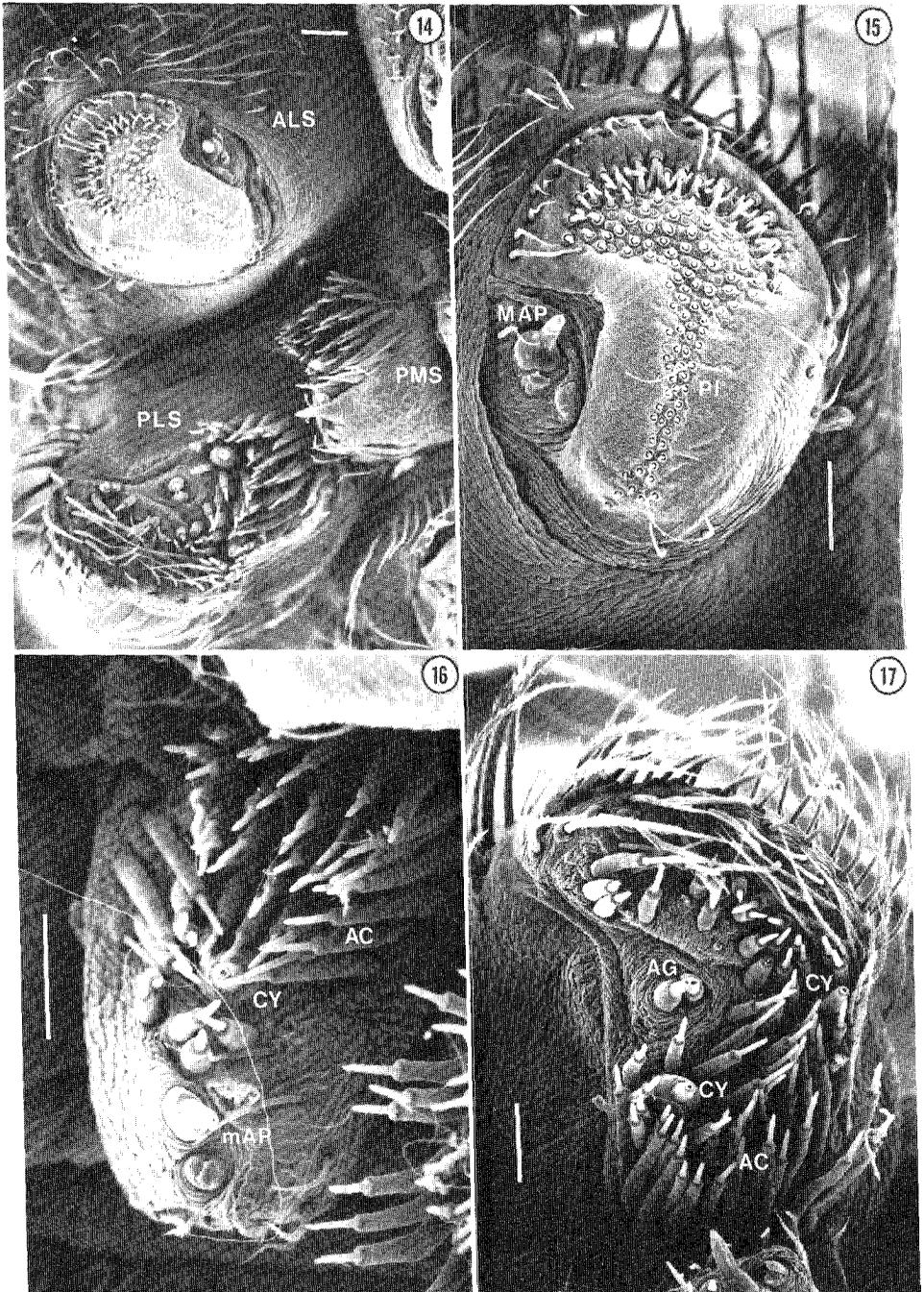
Figures 2-5.—*Deinopis spinosus* Marx spinnerets: 2, left spinneret group; 3, anterior lateral spinneret, closeup; 4, posterior median spinneret, closeup (subadult female); 5, posterior lateral spinneret, closeup. Abbreviations for Fig. 1-41: AC = aciniform; AG = aggregate; ALS = anterior lateral spinneret; FL = flagelliform; MAP = major ampullate; mAP = minor ampullate; CY = Cylindrical; PA = paracribellar; PF = pseudoflagelliform; PI = piriform; PMS = posterior median spinneret; PLS = posterior lateral spinneret. Scale bars = 50 μm.
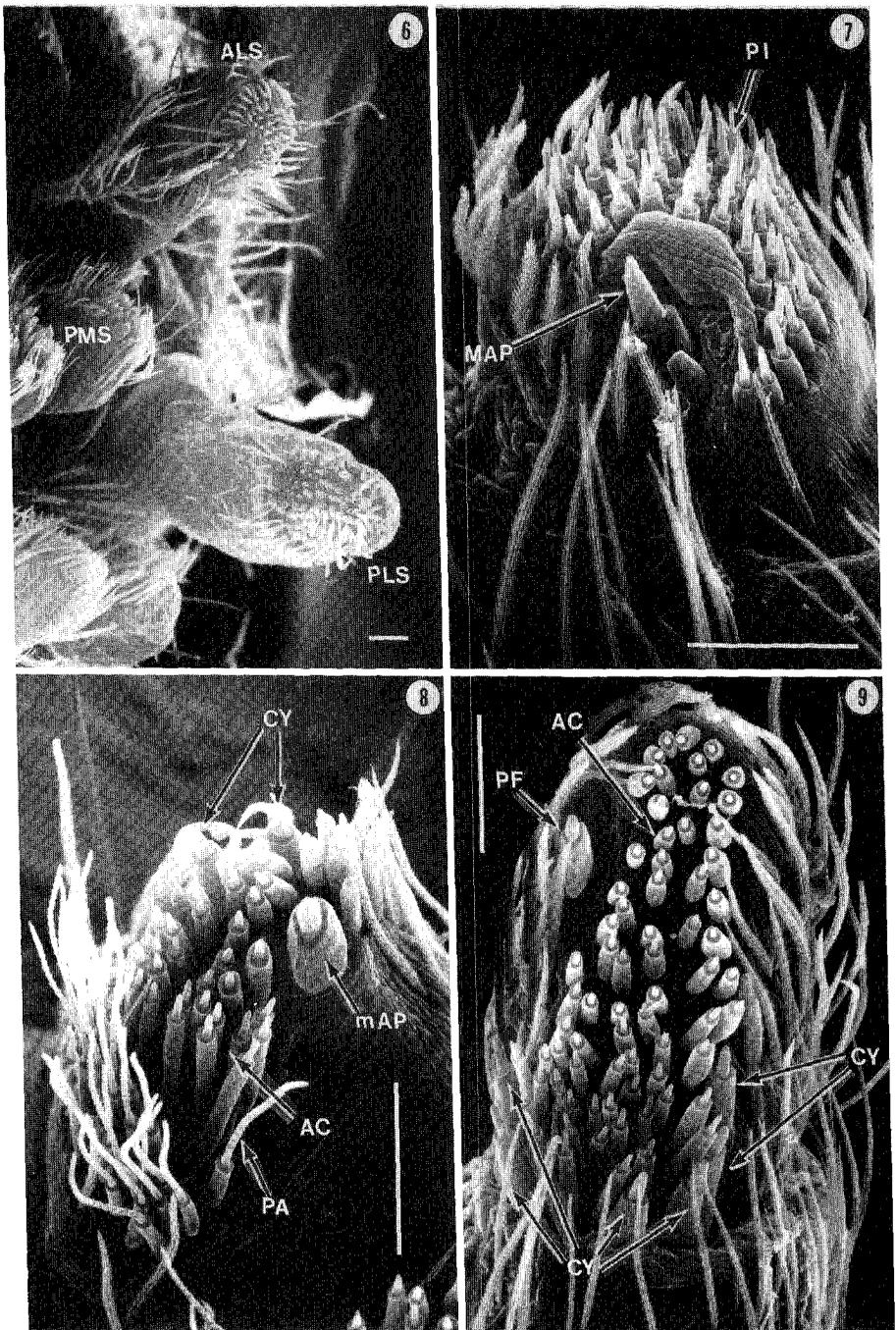
Figures 6-9.—*Octonoba octonarius* (Muma) spinnerets: 6, left spinneret group; 7, anterior lateral spinneret, closeup; 8, posterior median spinneret, closeup; 9, posterior lateral spinneret, closeup. Abbreviations as in Figs. 2-5. Scale bars = 50 μm.
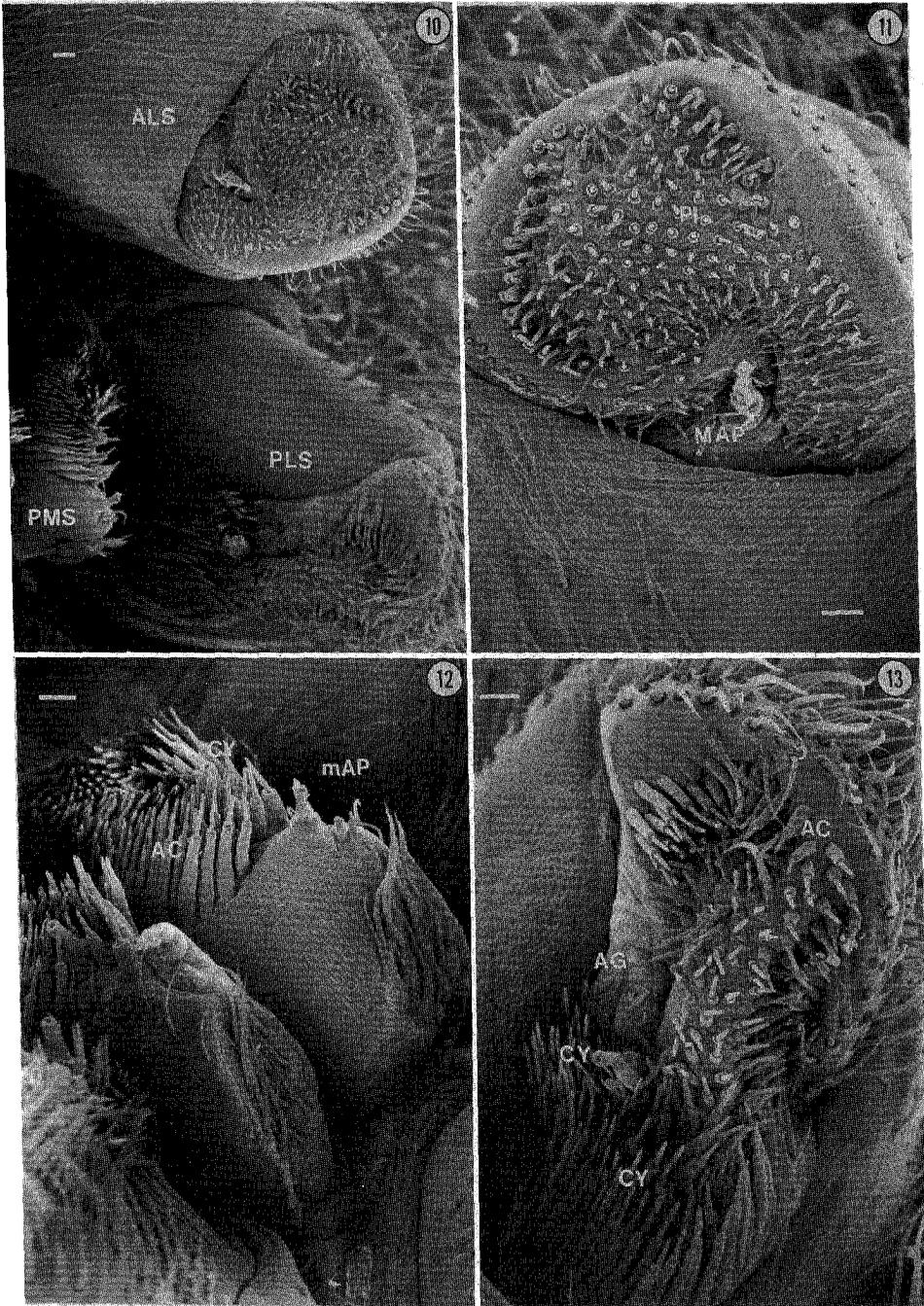
Figures 10-13.—*Araneus diadematus* Clerck spinnerets: 10, left spinneret group; 11, anterior lateral spinneret, closeup; 12, posterior median spinneret, closeup; 13, posterior lateral spinneret, closeup. Abbreviations as in Figs. 2-5. Scale bars = 50 μm.

Figures 14-17.—*Mecynogea lemniscata* (Walckenaer) spinnerets: 14, left spinneret group; 15, anterior lateral spinneret, closeup; 16, posterior median spinneret, closeup; 17, posterior lateral spinneret, closeup. Abbreviations as in Figs. 2-5. Scale bars = 50 μm.

aggregate spigots is not as pronounced (*Gaucelmus*, Fig. 37; *Nesticus* and *Eidmanella*, not figured). In addition, the aciniform spinning fields on the PMS are much reduced in comparison with araneids, and are limited to the posterior and apical surface of the spinnerets.

DISCUSSION

Spigot homologies.—The above results argue that by judicious use of spigot number, placement, appearance, and known ontogenetic patterns, one can often work out spigot homologies without reference to histological data. A basic rule of inference is the difference between morphological singulars and multiples, or homologues and homonoms (Riedl 1979). Morphological singulars are unique and can be exactly specified, such as "the left third tarsus", or, in this case, "the basal cylindrical gland of the PLS." On the other hand, morphological "multiples" are present in many copies and are not individually specifiable, such as "abdominal setae," or, in this case, the "aciniform spigots of the PMS." One can make exactly specifiable homology statements about morphological singulars, but usually one can only homologize groups or sets of morphological multiples. In the case of spigots one has both singulars, such as major ampullates, minor ampullates, cylindricals, pseudoflagelliforms, aggregates and flagelliforms, and "multiples," such as paracribellars, piriforms, and aciniforms. When multiple spigots are reduced in number, they may be consistent enough in their distributions to support hypotheses of individual homology (compare aciniform spigots in Figs. 4, 8, 12 vs. Figs. 28, 32, 36, 40). In combination with their placement, the distinction between singulars and multiples offers a way to tell the different kinds of spigots apart.

By trading off similarities between singular spigots, groups of spigots, and where they occur, one can devise a set of rules to guide homology statements about spigots, at least within the orbweavers. They can be summarized as follows:

MULTIPLES
   1. If the spigots are unique to the ALS and are small, numerous, and occupy most of the ectal spinning field, they are *piriform gland spigots*. They are used to attach draglines together or to the substrate. They should occur in all instars and both sexes.
   2. If they occur both on the PMS and PLS and are small, but slightly more elongate that the ALS piriforms, they are probably *aciniform gland spigots*. They have several uses: prey wrapping, retreats, and egg sacs. They should occur in all instars and both sexes.
   3. If they occur only on the PMS and are thin, very long, and with annulate shafts, they are probably *paracribellar spigots*. Their function is unclear, although they contribute a component to the sticky thread. They should occur in all instars and both sexes of all cribellate orbweavers, at least.

SINGULARS
   4. If a set of three spigots occur as two on the araneoid PLS and one on the anterior portion of the araneoid PMS, they are *cylindrical gland spigots*. They are apparently used to produce specialized silk for egg sacs. They
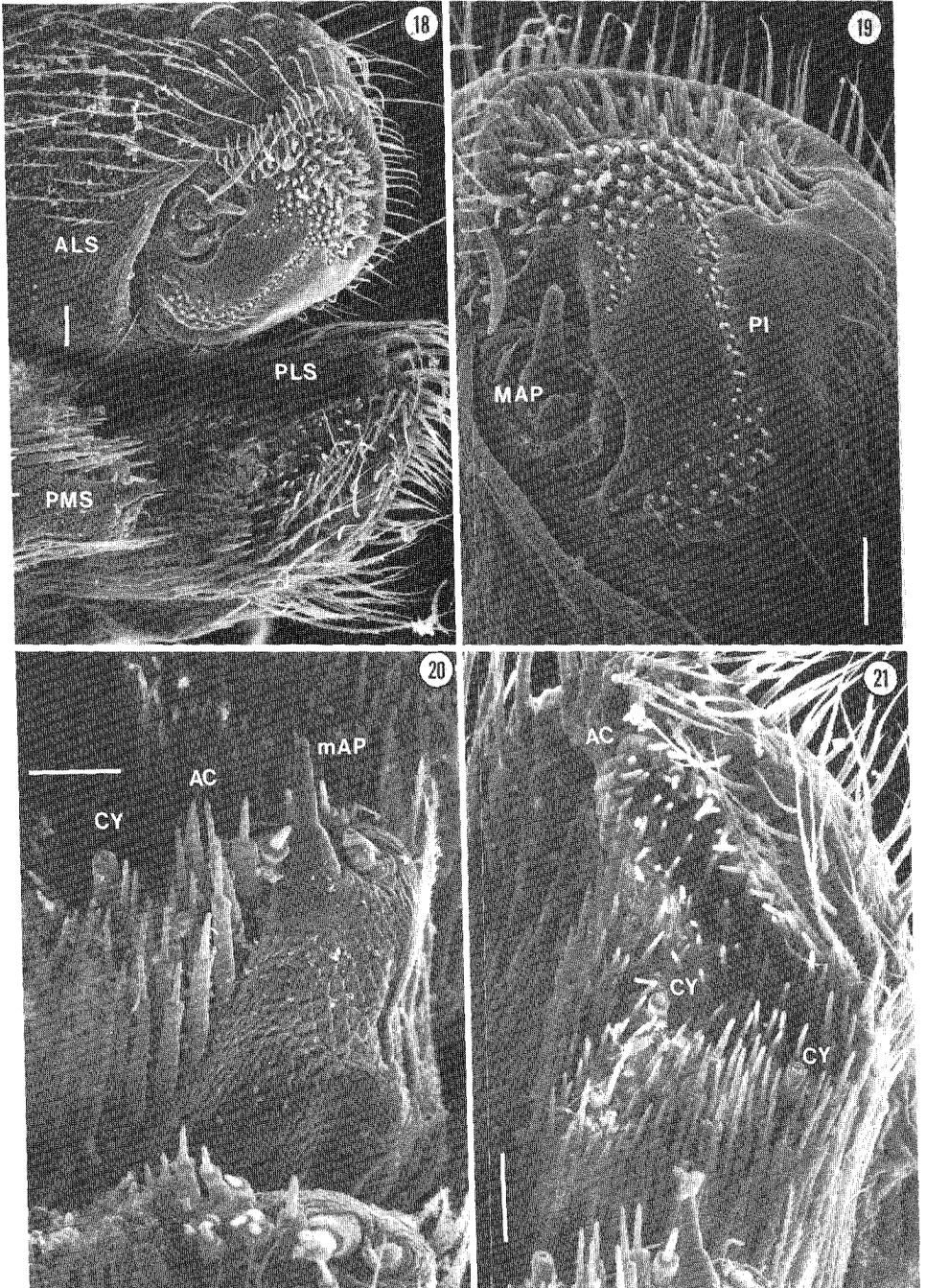
Figures 18-21.—*Cyrtophora citricola* (Forskål) spinnerets: 18, left spinneret group; 19, anterior lateral spinneret, closeup; 20, posterior median spinneret, closeup; 21, posterior lateral spinneret, closeup. Abbreviations as in Figs. 2-5. Scale bars = 50 μm.
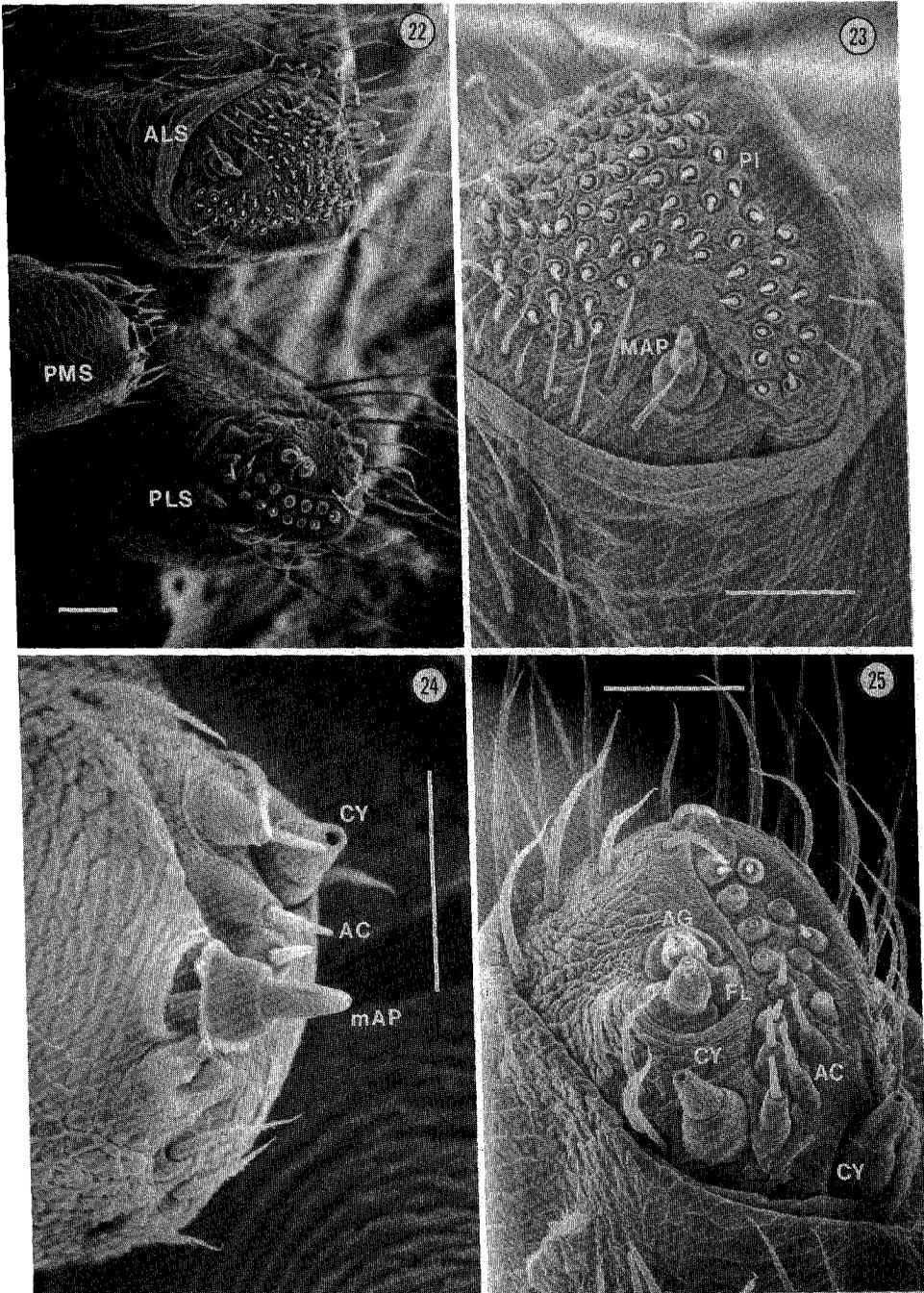
Figures 22-25.—*Leucauge venusta* (Walckenaer) spinnerets: 22, left spinneret group; 23, anterior lateral spinneret, closeup; 24, posterior median spinneret, closeup; 25, posterior lateral spinneret, closeup. Abbreviations as in Figs. 2-5. Scale bars = 50 μm.

should only occur in adult females. The situation in cribellate orbweavers is more complex because more cylindricals are present, but their distribution is as in araneoids.

5. If a pair (or group, Deinopidae) of spigots occur as one on the mesal margin of the ALS and one on the posterior margin of the PMS, they are *ampullate gland spigots*, usually one major and one minor. They are used for components of the dragline and major structural lines. They should occur in all instars and both sexes.

6. If a pair of large spigots with wide openings is juxtaposed on the anterolateral margin of the PLS, they are *aggregate gland spigots*, used to make the viscid glue of the sticky line. They should occur in juveniles and adult females, but are supposed to be absent in all adult males (exceptions may occur).

7. If a single large spigot is unique to the PLS anterolateral or apical margin, it is the *flagelliform* or *pseudoflagelliform gland spigot*, used to make the base fibers of the sticky line. Flagelliform spigots are usually between the two aggregate spigots, but not always. Like the aggregate gland spigots, flagelliform and pseudoflagelliform spigots should occur in juveniles and adult females, and should be absent in adult males.

These rules were used to identify the various spigots present in the genera illustrated here. Of these, only *Araneus, Latrodectus, Cyrtophora* and *Mecynogea* have been studied histologically (Kovoor 1972, 1977a, 1988; Kovoor and Lopez 1982, 1988), but no SEM illustrations of them have been published. As far as I know, only the spinnerets of *Nephila* among the araneoids have been comprehensively illustrated (Kovoor 1986), although details of spigots of several uloborids have been published (Peters and Kovoor 1980; Kovoor and Peters, 1988).

As an example one can use the above set of rules to correlate the large spigots on *Leucauge* PMS with known gland types. Because they are few in number, and are individually recognizable, they are probably examples of morphological singulars. Cylindrical glands exit on the PMS and PLS (#4, above). Araneoid ampullate glands exit only on the ALS and the PMS (#5, above). In Figs. 24-25, *Leucauge* has three large spigots with broad bases, sharply tapering, fluted, blunt shafts, and wide tips. The junction between the base and the shaft has a narrow indistinct rim. One of these spigots exits on the PMS (Fig. 24, CY), and two on the PLS (Fig. 25, CY). Therefore, they probably serve cylindrical glands.

Likewise, the major ampullate gland on the *Leucauge* ALS is similar to the spigot labelled as the minor ampullate gland on the PMS (Fig. 24, mAP). One can thus homologize spigots on orbweaving spiders, for example between *Leucauge*, a tetragnathid, and *Frontinella*, a linyphiid, even without histological evidence in these particular cases.

As an example of morphological multiples, piriform spigots are the only set of multiple spigots on the araneoid ALS tip. They are easy to recognize. However, in more distantly related groups, such as Deinopidae, two sets of multiples occur on the ALS. Only one set is on the mesal margin, set apart from the rest of the spinning field, and it is less numerous, and the spigots are larger. Because they occur in the same place as major ampullate glands, one can guess that they are indeed ampullate glands, and that Deinopidae is derived in having multiple ALS
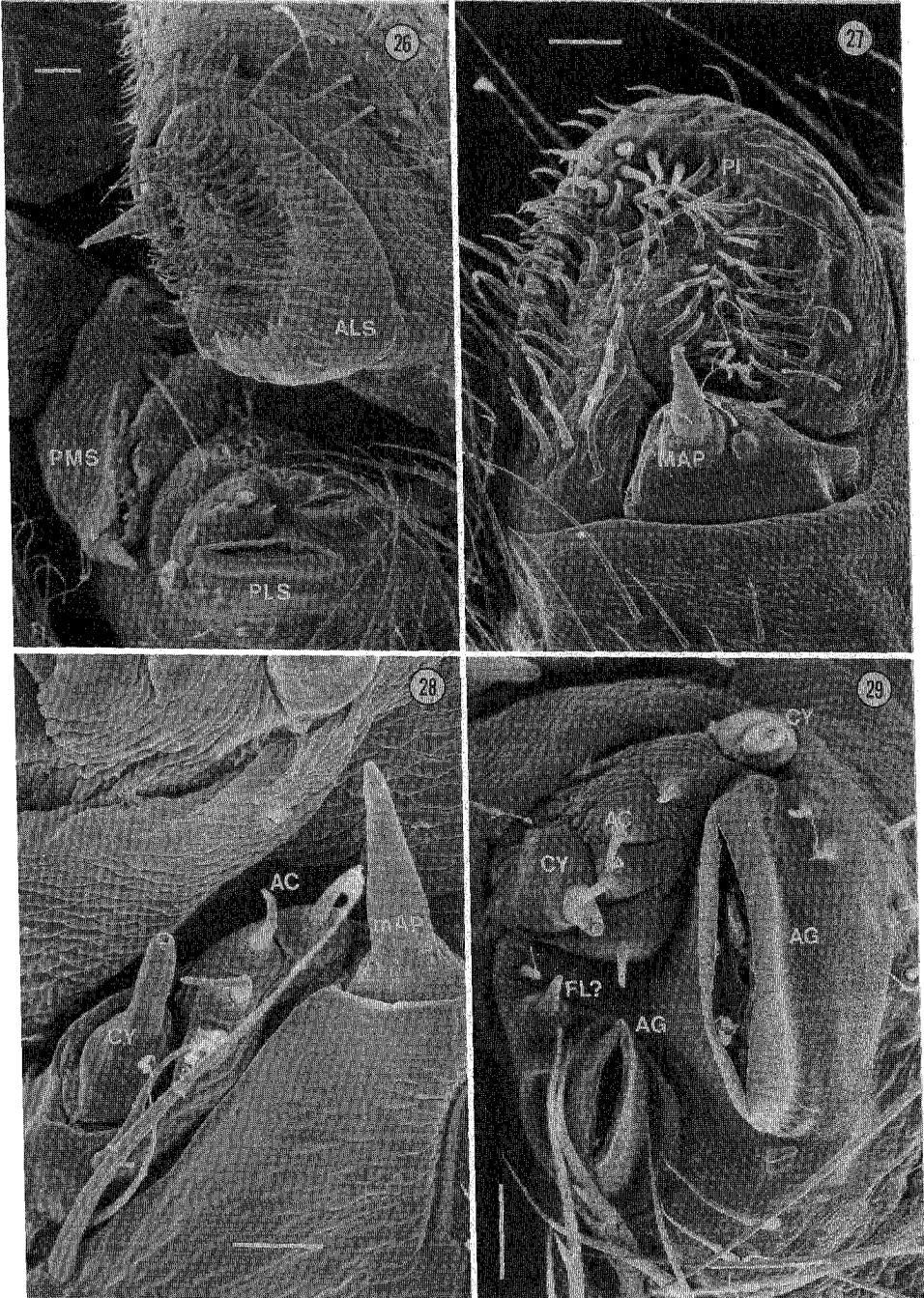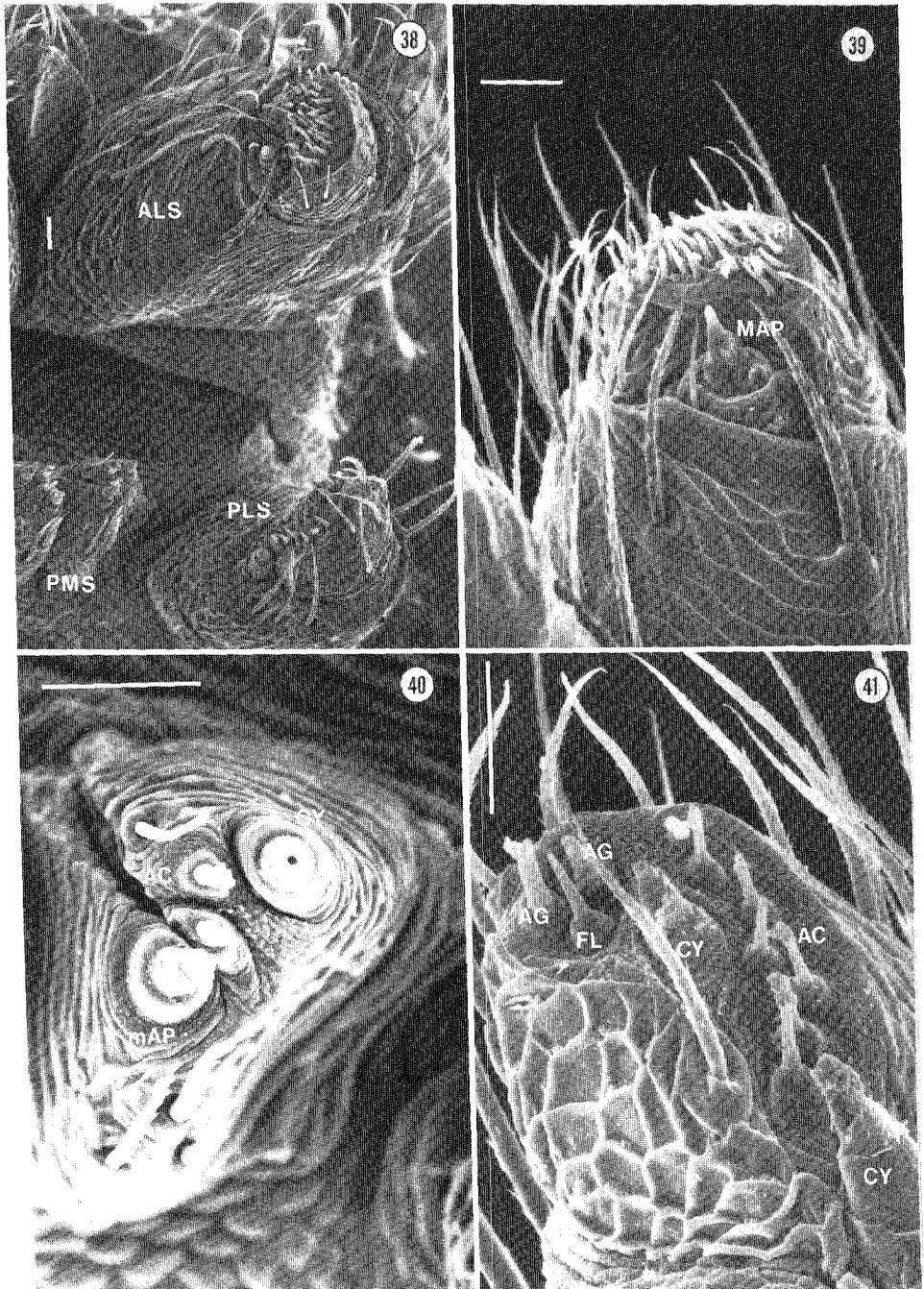
Figures 26-29.—*Latrodectus variolus* (Walckenaer) spinnerets: 26, left spinneret group; 27, anterior lateral spinneret, closeup; 28, posterior median spinneret, closeup; 29, posterior lateral spinneret, closeup. Abbreviations as in Figs. 2-5. Scale bars = 50 μm.
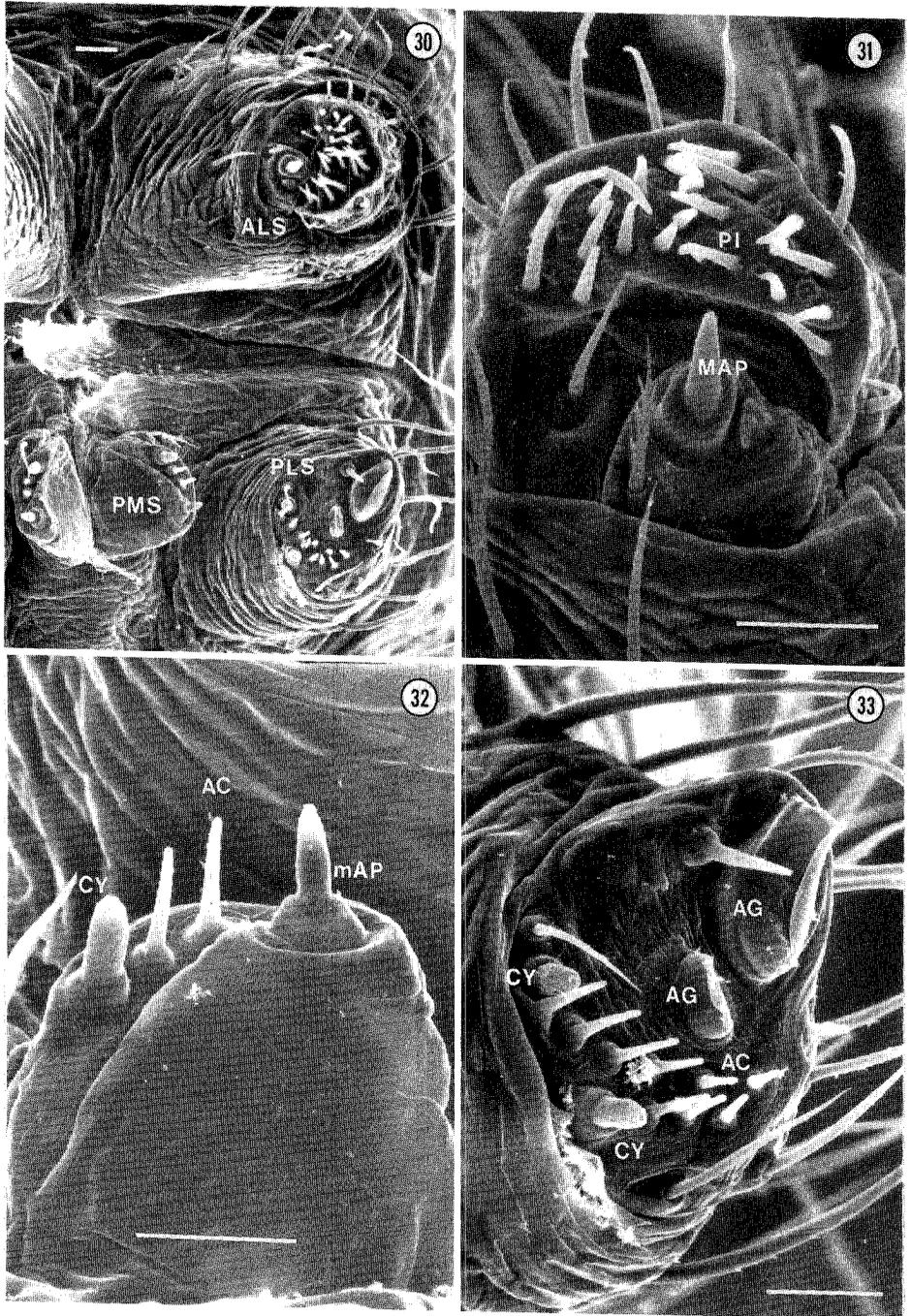
Figures 30-33.—*Theridula opulenta* (Walckenaer) spinnerets: 30, left spinneret group; 31, anterior lateral spinneret, closeup; 32, posterior median spinneret, closeup; 33, posterior lateral spinneret, closeup. Abbreviations as in Figs. 2-5. Scale bars = 20 μm.
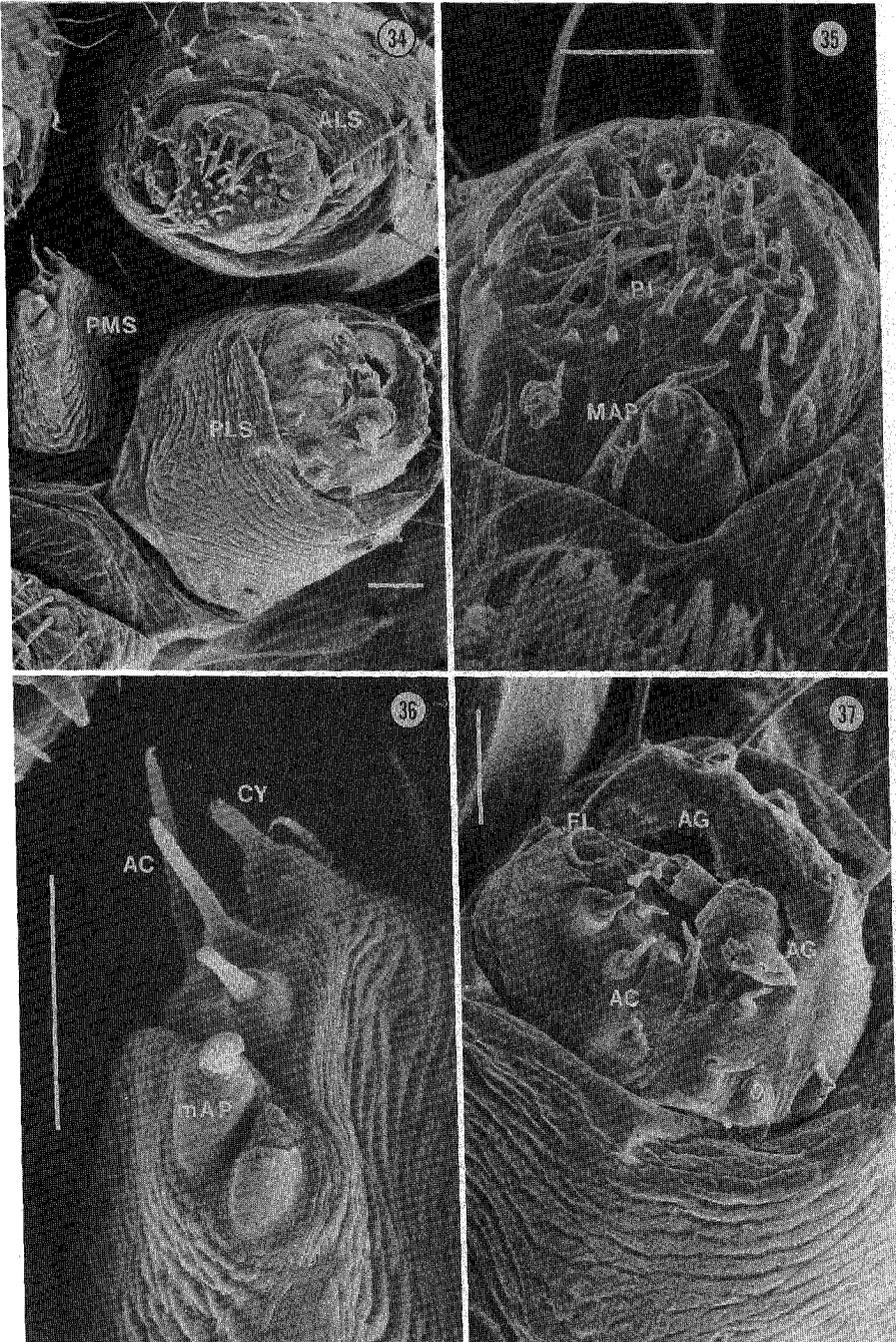
Figures 34-37.—*Gaucelmus angustinus* Keyserling spinnerets: 34, left spinneret group; 35, anterior lateral spinneret, closeup; 36, posterior median spinneret, closeup; 37, posterior lateral spinneret, closeup. Abbreviations as in Figs. 2-5. Scale bars = 50 μm.

Figures 38-41.—*Frontinella pyramitela* (Walckenaer) spinnerets: 38, left spinneret group; 39, anterior lateral spinneret, closeup; 40, posterior median spinneret, closeup; 41, posterior lateral spinneret, closeup. Abbreviations as in Figs. 2-5. Scale bars = 20 μm.

ampullate glands. This may be a synapomorphy for the family (it also occurs in *Menneus*, pers. obs.). By position and morphology, the other group of smaller multiples on the deinopid ALS are therefore the piriforms. By similar logic, one can also guess that deinopids are strange in having up to 50 pairs of cylindrical gland spigots (Fig. 4, CY). Although the histology of deinopid glands is unknown, one may predict the following peculiarities: 5-10 pairs of major ampullate glands, numerous cylindrical glands, and a single pair of pseudoflagelliform glands.

As a final example of spigot identification, the PMS of *Araneus* have three kinds of spigots: one kind of multiples and two kinds of singulars. The multiple spigots (Fig. 12; AC) are recognizably more like the multiple spigots of the PLS (Fig. 13; AC) than they are like the multiple kind on the ALS (Fig. 11; PI). Probably they are aciniforms. Histological data (Kovoor 1972) suggests that only one sort of gland present in numerous copies exits on both the PMS and PLS: the aciniform glands (#2, above). In this case histology and morphology are again concordant. Similarly, the single posterior spigot on the PMS (Fig. 12; mAP) is more like the single ALS spigot (Fig. 11; MAP), than it is like any single spigot on the PLS. The single anterior spigot on the PMS is more like the two basal spigots on the PLS than it is like other PLS or ALS spigots. Histological data confirms that Araneoidea have a single cylindrical spigot on the PMS and two on the PLS, and only a single minor ampullate gland on the PMS.

Interpretation of patterns.—Once over the hurdle of identifying spigots, one can look for interesting differences among taxa. For example, deinopoid piriform spigots have raised bases with rounded shoulders (Figs. 3, 7; PI), but araneoid piriform spigots have sunken bases and sharp rims. I have found that deinopoids resemble other cribellate araneomorphs, and thus the deinopoid condition is primitive, and the araneoid condition is derived, probably a synapomorphy for the superfamily.

Aciniform spigots usually are small, have longer bases, distinct rims at the base-shaft junction, and elongate, slow-tapering shafts with a fine tip. The PLS aciniform field in *Leucauge* is obviously modified by being narrowed and focused into more regular, elongate rows. I have found the same feature in *Tetragnatha, Pachygnatha*, and to a lesser extent in *Meta*, all "metine" genera. It also occurs in at least some linyphiids (e.g., *Frontinella*, Fig. 41). Perhaps it is a synapomorphy for the same group of derived araneoids defined by use of an inside first leg forward tap during sticky segment localization behavior (Coddington 1986a). The distribution of PMS aciniform spigots is also intriguing. In *Araneus, Mecynogea*, and *Cyrtophora* a brush of aciniform spigots on the anterior face of the PMS is present (Figs. 12, 16, 20; AC). In the same position, cribellate orbweavers such as *Deinopis* and *Octonoba* (Figs. 4, 8) have paracribellar spigots, but they also have an extensive anterior brush of aciniform spigots. This is also true, for example, of *Micrathena* and *Cyclosa* among the araneids, various other deinopids and uloborids, and potential cribellate outgroups to orbweavers such as amaurobiids. An extensive anterior PMS aciniform brush is probably a plesiomorphic feature. More derived araneoids, such as *Leucauge, Latrodectus, Theridula, Gaucelmus*, and *Frontinella* illustrated here, have no similar anterior aciniform brush. *Nephila* also lacks aciniform spigots in the same area (Kovoor 1986; pers. obs.). The trait is also present in Anapidae, Theridiosomatidae, Mysmenidae, and other theridiids, nesticids, tetragnathids and linyphiids not illustrated here. Perhaps it is

related to increasing specialization of the spinning apparatus. Kovoor and Peters (1988) noted that histologically two classes of aciniform glands exist, aciniform A and aciniform B. I find that distinguishing their spigots with SEM is difficult, but, based on the uloborid *Polenecia*, they suggested that aciniform B spigots were somewhat larger. Interestingly, they also point out that linyphiids, metines, and theridiids (at least) among the araneoids lack aciniform A glands. Very possibly the reduced PMS aciniform field which can be seen with the SEM is the external morphological evidence for the lack of aciniform A glands among derived araneoids. If so, a reduced complement of PMS glands and spigots becomes an additional synapomorphy of derived araneoids (see Coddington 1986a for others), and is the first evidence for a more exact placement of theridiid-nesticids among the araneoids.

The reduction and focusing of aciniform spigots on both the PMS and PLS in derived araneoids correlates well with the absence (or reduction) of prey-wrapping behavior. Aciniform spigots probably are mostly responsible for the threads used in prey-wrapping (Table 1). As intimated previously (Coddington 1986a), prey-wrapping and in particular attack-wrapping seems to be a plesiomorphic feature of orbweavers that has been lost in the more derived lineages. This interpretation, which follows directly from cladistic reasoning and outgroup comparison, contradicts previous hypotheses about the evolutionary history of prey-wrapping that were based on the assumed adaptive value of the trait (Robinson 1975; Eberhard 1982). It also illustrates how adaptive hypotheses formed in the absence of cladistic information can mislead (Coddington 1988).

Some patterns are harder to explain. For example, *Araneus, Mecynogea, Cyrtophora, Leucauge,* and *Gaucelmus* all have what appears to be a vestigial spigot on the PMS, posterior to the mAP spigot (Figs. 12, 16, 20, 24, 36). It is probably the vestigial remnant of a minor ampullate gland spigot which is lost in the adult instar (like the vestigial ALS major ampullate, e.g., Figs. 1, 15). This nubbin is absent in *Theridula, Latrodectus, Frontinella,* and the cribellates. With this distribution, the feature might be another araneoid synapomorphy, uniquely lost in theridiids, or a synapomorphy of theridiids plus linyphiids, but one would need more evidence to say.

The data presented here also bear on a question in the spinneret histology literature. Based on histological evidence, Kovoor (1977c, 1978) was unsure whether the uloborid pseudoflagelliform gland was homologous to the araneoid flagelliform gland. They are apparently similar in shape, anatomy, and chemistry. Micrographs show that orb weavers all have a distinctive PLS spigot on the anterior margin. The morphology and placement of the spigot strongly suggest an interpretation of homology.

Nevertheless, Kovoor and Peters (1988) recently denied homology between flagelliform and pseudoflagelliform glands (and presumably their spigots), and also the monophyly of orbweavers. However, their arguments, when closely analyzed, misconstrue accepted rules of phylogenetic inference. They assert only that araneoids possess features not found in Deinopoidea (aggregate glands—a autapomorphy of Araneoidea); that Deinopoidea possess some features not found in araneoids (the cribellum, calamistrum, and paracribellum—plesiomorphies found in many cribellate taxa); and they imply that the pseudoflagelliform and flagelliform glands and spigots "cannot be regarded as homologous." The first two assertions are irrelevant to the problem at hand because they refer to an

autapomorphy and a plesiomorphy, respectively, and they unfortunately do not detail their evidence for the last assertion. On the other hand, they admit the many behavioral and web-architectural similarities between cribellate and ecribellate orbweavers, and presumably acknowledge the additional morphological similarities (Coddington 1986a). Given this suggestion of monophyly, and the lack of any evidence that links deinopoids or araneoids to a non-orbweaving group, most phylogeneticists would accept Hennig's principle that features should be regarded as homologues unless contradictory evidence overrules that inference. Put another way, one accepts the homology of bird wings and mammal forelimbs not because the differences between the structures are small or large, but because we have no evidence to contest the inclusion of birds and mammals in tetrapod amniotes. Exactly the same situation obtains in the case of the Orbiculariae (= Deinopoidea plus Araneoidea).

If both spigot morphology and gland histology agree, as in the issues of identification discussed above, then homology statements are doubly strong. If one source of evidence is suggestive but ambiguous, the other may resolve the issue, as for pseudoflagelliform and flagelliform glands. If actual conflicts in synapomorphy schemes exist, however, it would be difficult to decide with complete objectivity which source to accept, especially since we do not have much experience in evaluating for phylogeny either gross spigot morphology, or histochemistry and gland ultrastructure.

Comparison with histological and behavioral data.—It is interesting that a surprisingly high number of histochemical and ultrastructural facts have not been concordant with other comparative data on spiders, and thus with inferred phylogenies. Some strange examples are: an S-shaped major ampullate gland is characteristic of Hersiliidae and Nephilinae (Kovoor 1987); the proximal part of the ampullate gland is reduced to a collar of cells in, e.g., *Hypochilus, Filistata, Dictyna, Amaurobius, Telema, Pholcus, Uroctea,* and *Linyphia* (but not in *Oecobius* or other araneoids); three secretory regions are only present in the ampullate glands of *Cyrtophora, Cyclosa,* and *Gasteracantha* (not a monophyletic group), but only two in remaining araneids; pyriform glands are tripartite in *Leucauge* and *Oecobius,* but unipartite in *Uroctea* and, presumably, other metines; *Hersilia,* but not *Oecobius* or *Uroctea,* has tripartite aciniform glands; amino terminal groups are present in aciniform glands of theridiids and linyphiids, which apparently correspond to the aciniform B glands of Araneidae, Hersiliidae, and *Polenecia* uniquely among the uloborids (most examples from summary in Kovoor 1987). Any biologist familiar with corroborated phylogenies of spiders would be puzzled, to say the least, by the above groupings.

I am not sure why this is so. It may be because histochemical analysis often focuses on the chemical *behavior* of molecules, and not on their informational structure. The same lack of concordance with other systematic information was apparent in the early biochemical analysis of enzymes that gave, for example, percentages of specific amino acids, pH data, or molecular weights. Only later did biochemists discover that the informational content of enzymes was in the sequence of amino acids, rather than their relative abundances or other such summary features. Likewise, chemical characterizations of glandular products, for example as "acidophilic", "tyrosine-rich", "carboxyl-rich", or "rich in reducing groups" simply may not identify conservative phylogenetic features. While realizing that the phylogenetic analysis of these ultrastructural characters is still

young, at this point it is clear that observed points of similarity in some cases contradict massively corroborated phylogenetic groupings. Therefore homology arguments based on histochemistry and ultrastructure apparently need careful evaluation.

Whether the same difficulties of interpretation will characterize the study of gross morphology and distribution of spigots remains to be seen. Thus far the best known groups are the araneoids, and their spigot distributions are apparently more or less concordant with other phylogenetically useful character systems.

Spinning behavior obviously must depend to some extent on spinnerets and spigots. Therefore it is appropriate to comment also on recent behavioral research. Eberhard (1987) recently studied aspects of cribellate web-building behavior in what are apparently primitively non-orbweaving groups. He concluded that the tendency to spin sticky silk centripetally in cribellate and ecribellate orbweavers was widespread and probably plesiomorphic. That is, other non-orbweaving cribellate taxa such as filistatids, eresids, psechrids, and dictynids also start the spinning of sticky silk at the edge of their webs, and finish at the center or at the retreat. A centripetal tendency in the spinning of cribellate silk outside the true orbweavers was one specific prediction of the monophyly hypothesis (Coddington 1986a: 362). The fact that it occurs in the entire range of cribellate taxa is disappointing because so broad a distribution offers no evidence as to which of these taxa is the sister group of orbweavers. On the other hand, it partially overlaps the distribution of the pseudoflagelliform and paracribellar spigots, and thus all of these features when analyzed in tandem may elucidate araneomorph phylogeny.

In contrast, Eberhard (1987) argued that this widespread behavioral trait made it more plausible that behaviors characteristic of orb-weaving had evolved at least twice. In contrast, I still see no evidence that these behaviors are convergent. As pointed out above, such a conclusion would be logical only if synapomorphic features were discovered that linked only a portion of the orbweavers with a primitively non-orbweaving group.

Of course orbweavers, whether cribellate or not, still exhibit many unique and detailed behaviors, such as laying sticky silk in a continuous spiral, shifting combing legs (cribellates) or plucking-snubbing legs (araneoids) halfway through construction of a single sticky segment, sticky spiral localization, frame behavior, exploration behavior, non-sticky spiral construction, and the over-all algorithm of web construction. Orbweavers are also distinctive among all other major groups of spiders because complete and typical webs are produced in a single behavioral bout, usually lasting a few hours or less. Other web-spinning spiders typically take several days, and several bouts of behavior, to complete the architecture typical of their taxon. The behaviors unique to orbweavers are similar not only in gross aspect and function, but also in the details of movements of individual legs. Many of these appear to be true behavioral synapomorphies for orbweavers. The tendency to lay sticky silk centripetally is all very well, and it may even define a monophyletic group (however huge, if it includes everything from Filistatidae to Araneoidea). Indeed it seems possible that the centripetal tendency in sticky silk spinning may even be a primitive trait of Araneomorphae.

Interestingly, the same sort of conclusion apply to the pseudoflagelliform gland. Kovoor (1987) mentions glands "identified as pseudoflagelliform" for eresids, amaurobiids, psechrids, and zoropsid spiders. She also mentions that such glands

are not found in Filistatidae or, surprisingly, in Dictynidae. Histological observations on Hypochilidae, and Austrochilidae have not been published, but I have found several distinctly shaped spigots on the PLS of filistatids, hypochilids, and austrochilids, as well as amaurobiids and eresids. Given the position of flagelliform gland spigots in araneoids and pseudoflagelliform spigots among deinopoids, one would expect to find homologues of these glands uniquely on the PLS. On the other hand, I have not yet found any specialized morphologies in eresids or amaurobiids, and thus something of a contradiction is developing between histological and morphological pattern. Regardless of how this smaller controversy is resolved, if the histological research accurately identifies homologues of the uloborid pseudoflagelliform gland, we may have to conclude that primitive pseudoflagelliform glands and spigots evolved soon after the origin of araneomorph spiders—at least soon after the origin of the Araneoclada (all araneomorphs exclusive of Hypochilidae, Austrochilidae, and Gradungulidae).

Like a "centripetal tendency" in sticky silk spinning, then, the pseudoflagelliform gland would become part of the primitive ground plan for most true spiders. Arguments for the unique homology of the deinopoid and araneoid condition would then depend on further special similarities such as that the single pair of glands opens only on the PLS, and that they provide the sole pair of base fibers of the sticky line in both cases.

Conclusions.—Taken together, the spigot evidence thus far corroborates the hypothesis of orbweaver monophyly, and certainly does not dispute it. The spinning fields of cribellate orbweavers are more similar to those of ecribellate orbweavers than they are to non-orbweaving cribellate groups. Among araneoids, the Araneidae still exhibit fairly primitive spinneret morphologies. The same can be said for the details of web construction (Coddington 1986a, b). As always, being primitive in one feature does not make a taxon primitive in all respects. Thus araneid genitalia may be relatively more derived than those of metines or the symphytognathoid taxa, or at least far from the orbweaver ground plan.

All of the spiders considered here are orbweavers, or are descended from orbweavers, based on other evidence. Although this character system is obviously useful within the orbweavers, it would be interesting to know how well this system of logic will work for more distantly related, and less derived cribellate groups which may be the sister taxon to orbweavers, such as Hypochiloidea, Amaurobioidea, or Dictynoidea. Even though among distantly related and little studied groups the use of the conventional names for araneoid glands becomes increasingly risky and less justifiable, the basic method of comparing between singulars and multiples, between sexes and instars, and from one spinneret to another should be a primary tool for deciphering spigot homology.

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